Quantitative imaging of the collective cell movements shaping an embryo

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The recent development of imaging and image processing techniques, such as 4D microscopy and 3D cell tracking, enables analysis through quantification of the movement of large cell populations *in vivo*. These imaging approaches provide an opportunity to study embryonic morphogenesis during development from the level of cellular processes to the scale of entire organism. Image analysis reveals cell collective behaviors that shape an embryo and offers some surprising insights into the cell-cell interactions involved in concerted movements. We illustrate the power of this approach by studying the early development of *Drosophila* embryos.

I. INTRODUCTION

Many cellular processes involved in embryonic morphogenesis are stereotypical and occur at different stages, in different tissues or species with similar characteristics. Such stereotypical events include collective cell migration, tissue invagination, or spatio-temporal patterns of cell division. Developing systematic and standard methods to quantify these morphogenetic events combining *in vivo* imaging and computational analyses are powerful tools to help our understanding of how embryos are shaped and how a single cell can develop into a complete organism. We focused our study on gastrulation, a universal event that convert early embryos from a ball or a sheet of cells into a multilayered structure, composed of mesoderm, ectoderm, and endoderm [1]. At this stage of development, *Drosophila melanogaster* embryos have ~6000 cells that undergo extensive morphogenetic movements. These movements are extremely reproducible and regulated in space and time. One of them is the formation of the mesoderm layer. A few minutes after the invagination of ~800 mesoderm cells on the ventral side of the embryo to form a tube (Fig. 1A), this tube collapses (Fig. 1B) and, during the 2 hours that follow, the cells migrate to form a monolayer at the surface of the ectoderm epithelium (Fig. 1C). Here we present a quantitative strategy based on multiphoton microscopy, image processing and computational analysis to follow the dynamics of a large cell population and to study collective cell migration. We recently applied this strategy to study the role of Fibroblast Growth Factor (FGF) signaling in the control of mesoderm spreading [2].

II. IMAGE ACQUISITION AND 3D-CELL TRACKING

Two-photon excited fluorescence microscopy is particularly well adapted for imaging scattering tissues, such as early

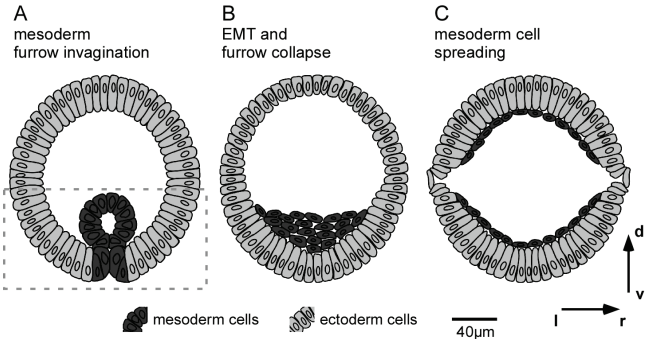

Fig. 1. The three steps of mesoderm formation during *Drosophila* gastrulation. (A) ventral furrow formation, (B) epithelial-mesenchymal transition (EMT) and furrow collapse (C) mesoderm cell spreading. The dashed box in A indicates the field of view of the 3D image represented in Fig. 3. Posterior view. d: dorsal, v: ventral, l: left, r: right.

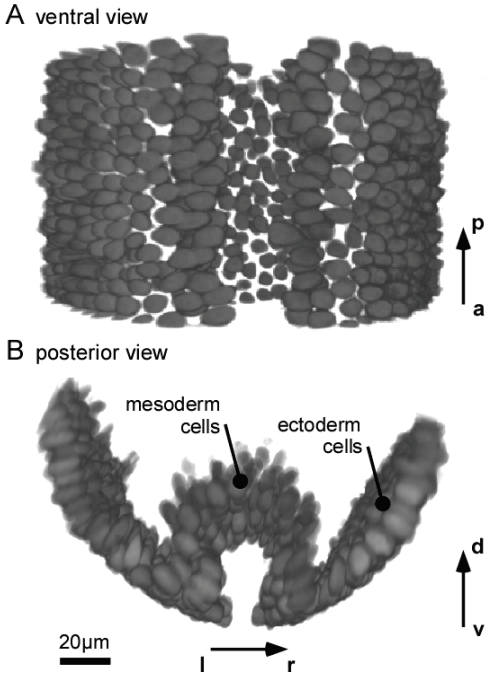

Fig. 2. 3D image reconstruction of the distribution of GFP-labeled nuclei within a *Drosophila* embryo during gastrulation at the onset of mesoderm spreading. The ventral (A) and dorsal (B) views show the two cell populations: the internalized mesoderm cells forming the ventral furrow and the ectoderm cell layer. a: anteriror, p: posterior, d: dorsal, v: ventral, l: left, r: right.

Drosophila embryos, with limited phototoxicity [3]. This technique enables the imaging of the deepest mesoderm cells after internalization (Fig. 2), which is not possible using other fluorescence microscopy techniques, such as confocal microscopy. Up to 2,000 cells were imaged in 3D during 2-3 hours of development with 45 second time resolution and 0,5μm x 0,5μm x 1μm voxel size. Each imaging dataset encompassed typically ~$3*10^9$ voxels. Up to 100,000 cell positions were identified by segmenting the fluorescence signal from the nuclei (Fig. 3). The segmentation was performed in 3D using the spot detection of Imaris and was manually corrected. Finally, the trajectories of both mesoderm and ectoderm cells were quantified by tracking in time the segmented nuclei using Imaris.

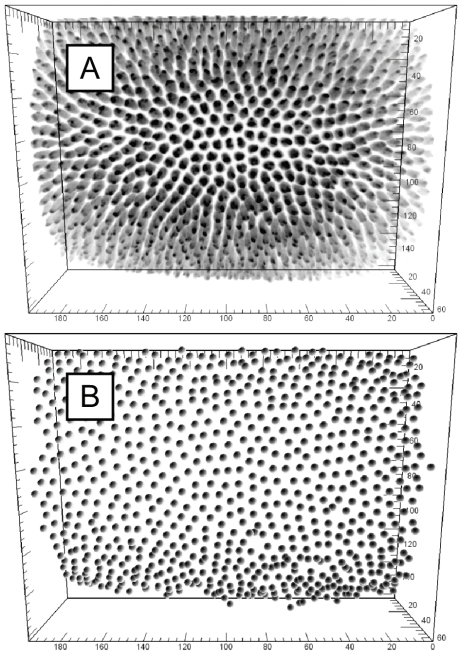

Fig. 3. Automatic segmentation of >1000 nuclei in *Drosophila* at the onset of gastrulation. The fluorescent signal from GFP (A) is segmented in 3D and each spot in (B) represents the position of the nuclei. Scale bars in μm.

III. TEMPORAL AND SPATIAL REGISTRATION

In order to compare the cell movements from one embryo to the other, the cell-tracking datasets were registered in time and space. First, the position of each cell was defined in accordance with the body plan using a cylindrical coordinate system, which is better adapted to the shape of the embryo (Fig. 2 and Fig. 3A). A cylinder was fitted to the 3D distribution of ectoderm cells. The main axis of the fitted cylinder corresponds to the anterior-posterior (AP) axis of the embryo. In the resulting cylindrical coordinate system, each direction corresponds to a specific morphogenetic event. Hence, the complex cell movements in 3D can be decomposed into their components along the radial, angular and longitudinal directions, which facilitate their study [2]. The spatial origin in the angular direction was defined as the position of the midline cells. These cells are aligned in the most ventral part of the ectoderm. Finally, the datasets where synchronized in time by defining t=0 as the onset of ectoderm movements in the AP direction. This movement occurs in every embryo and is not disrupted in the mutants.

IV. MECHANICAL COUPLING BETWEEN CELL LAYERS

During gastrulation, both ectoderm and mesoderm cells move simultaneously. As mesoderm cells migrate on top of the ectoderm layer, we investigated the mechanical coupling between the two cell populations. The local correlation of the velocity components in each direction revealed that their movements are strongly correlated in the AP direction and not the others [2]. We assumed that the strong movement in the AP direction is due to the well-described ectoderm movement of germ-band extension. In order to visualize the residual movement of mesoderm cells, the average displacement of neighboring ectoderm cells was substracted from the displacement of each mesoderm cell. The result presented in Fig. 4 confirms the correlation analysis: the component in the AP direction, describing the residual displacement of mesoderm cells, is drastically reduced. This result has several implications: (I) the relative migration of mesoderm cells on top of the ectoderm layer is directed in the angular and radial directions, (ii) the study of this migration can be limited to two dimensions, which significantly simplifies the analysis, (iii) the uncoupling of mesoderm and ectoderm movements by processing the tracking data enable to investigate mutants exhibiting a disruption of both mesoderm and ectoderm movements.

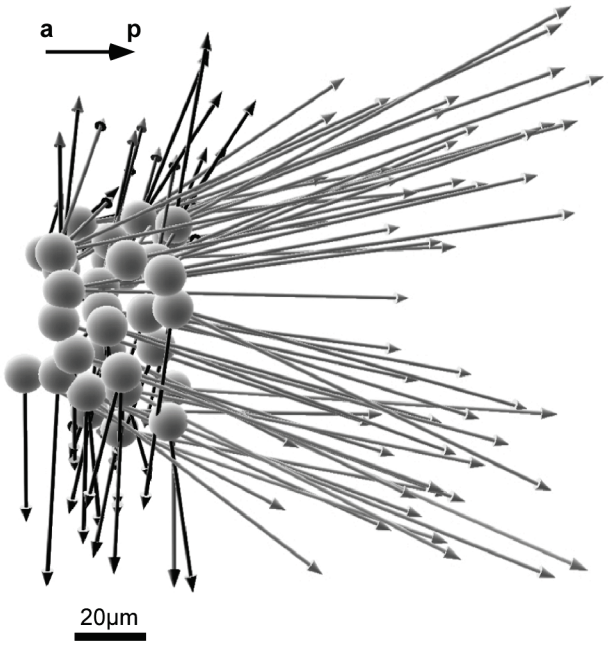

Fig. 4. Displacement of a selection of mesoderm cells before (black arrows) and after (gray arrows) removing the local average displacement of ectoderm cells. The residual displacement of mesoderm cells is mainly directed perpendicular to the anterior-posterior axis. a: anterior, p: posterior.

V. ANALYSIS OF MORPHOGENETIC EVENTS

This systematic decomposition of cell movements allowed us to investigate the complex sequence of events involved in mesoderm formation. Several processes were quantified from the 3D tracking datasets, such as the patterns of cell movements, of cell division and of cell intercalation. This analysis revealed that mesoderm formation in wild-type (*wt*) embryos exhibits highly organized collective cell migration, waves of cell division and synchronized cell intercalation, as reported in [2]. Finally, this approach allowed us to investigate the subtle phenotype of FGF mutant embryos: a statistical analysis of the mesoderm cell migration in the angular direction demonstrated that only a sub-population of mutant cells are disrupted in their ability to move in a directed manner; whereas, the other sub-population shared the same collective behavior as observed in the *wt* embryos [2]. This surprising result provides new insights into the role of FGF signaling and demonstrated the power of such a quantitative approach to dissect complex morphogenetic events into their cellular and molecular components.

V. CONCLUSION

The combination of 4D imaging using multiphoton microscopy, 3D-cell tracking and computational analysis provided a powerful approach to study the mesoderm formation in *Drosophila* embryos. Several straightforward methods were combined to facilitate the investigation of embryonic morphogenesis: the choice of a spatial coordinate system adapted to the geometry of the process(es), the registration of both spatial and temporal information regarding movements of cells, the decomposition of cell displacements into their spatial components, and the reduction of the number of dimensions describing the process to the minimum required for a proper analysis. More generally, the systematic analysis of morphogenetic events developed for this study should be applicable to other model systems.

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